

AMENDMENTS TO THE SPECIFICATION

Please replace the 3rd full paragraph on page 9 with the following amended paragraph:

The protoporphyrinogen oxidase gene can be cloned, by introducing the expression type cDNA library into an *E. coli* mutant strain in which *hemG* (protoporphyrinogen oxidase gene) is deleted, and isolating a cDNA clone which complements the growth. The whole DNA sequence of the gene thus cloned can be determined by Maxiam-Gilbert method or Sanger method. Further, the search for protein ~~design~~coding region, and the analysis of homology of the nucleotide sequence with known genes can be performed by using a commercially available software for analysis of a nucleotide sequence, for example, Genetyx GENETYX (SDC Co. Ltd.) or DNASIS (Hitachi software engineering Co., Ltd.).

Please replace the 2nd full paragraph on page 17 with the following amended paragraph:

Preparation of cDNA library was conducted by using Superscript Lambda System SUPERSCRIPT LAMBDA SYSTEM cDNA library construction kit (manufactured by GIBCO BRL Co., Ltd.). By using this kit, cDNA can be inserted at the positive orientation against lacZ promoter of λgt22A, by using a primer-adaptor at the first strain synthesis.

Please replace the 3rd full paragraph on page 17 with the following amended paragraph:

4 μg of mRNA was used as a template for cDNA-~~sunthesissynthesis~~ synthesis. cDNA first strand synthesis, cDNA second strand synthesis, ligation of adaptor, restriction enzyme digestion and

column chromatography were conducted according to the attached manual. cDNA thus obtained was ligated with λ gt22A, and phage particle was reconstructed using ~~Gigapack Gold~~ GIGAPACK GOLD packaging kit (manufactured by Stragene Co., Ltd.), according to the attached manual. The primary library containing about one million and nine hundred thousand of independent clones were infected with *E. coli* Y1090 (r-) strain, multiplies and stored as an amplification library.

Please replace the 2nd full paragraph on page 19 that continues onto page 20 with the following amended paragraph:

From the pBNtPX-1 which recovered the poor growth of *E. coli* SASX38 strain, deletion clones were produced by using Deletion Kit for Kilo-Sequence (manufactured by Takara Shuzo Co., Ltd.), according to the attached manual, and the nucleotide sequence and amino acid sequence in the open reading frame were analyzed by using Cycle Sequencing Kit ~~AmpliTaq FS~~ AMPLITAQ-FS (manufactured by Perkin-Elmer Corporation) and an auto sequencer (manufactured by ABI PRISM 310, Perkin-Elmer Corporation) according to the attached manual. The obtained nucleotide sequence and the amino acid sequence in the open reading frame are shown in the SEQ ID No. 1. By using ~~Genetyx~~ GENETYX (SDC Co., Ltd.), gene analysis software, nucleotide sequence and amino acid sequence were compared with those of the protoporphyrinogen oxidase of *Arabidopsis thaliana* which have already reported (WO 95/34659), and the homology was examined. As a result, pBNtPX-1 showed high homology of 69 % at nucleotide level and 76 % at amino acid level with the chloroplast-type

protoporphyrinogen oxidase cDNA of *Arabidopsis thaliana*, and considered to be a chloroplast-type protoporphyrinogen oxidase cDNA of tobacco.

Please replace the 3rd paragraph on page 20 with the following amended paragraph:

Table 5 Comparison of amino acid sequence of tobacco (SEQ. ID. NO. 2) (upper column) and *Arabidopsis thaliana* (SEQ. ID. NO. 11) (lower column) of a chloroplast-type protoporphyrinogen oxidase

Please amend the 1st paragraph on page 21 that continues onto page 22 as follows:

After extracting the total RNA by the SDS/phenol method from photobleaching herbicide tolerant callus, ETR-245 cell line, and sensitive cell line, respectively, mRNA was purified by mRNA purification Kit (manufactured by Pharmacia Co., Ltd.). Reverse transcription reaction was conducted using 1 µg of the purified mRNA as a template and using Oligo(dT)12-18 (manufactured by Life Technology Oriental Inc.) as a primer and using

Superscript**SUPERSCRIPT™** RNaseH-Reverse Transcriptase (manufactured by Life Technology Oriental Inc.) according to the attached manual at 37 °C for 1 hour, and then cDNA was purified by phenol/chloroform extraction and ethanol precipitation. Protoporphyrinogen oxidase cDNA was attempted to be amplified by PCR, using the obtained cDNA as a template. A forward primer (5'-GCG GTC TAC AAG TCA GGC AGT CAT-3', SEQ ID No.6) and a reverse primer(5'-CAT GCC AAT TTT CCC AAG GCA TGA TCG TAT T-3', SEQ ID No.7) were used as the primer for PCR. One cycle of 94 °C for 3 minutes, and 30 cycles of 94 °C for 20 seconds, 61 °C for 30 seconds and 72 °C for 1 minute and 30 seconds and one cycle of 72 °C for 5

minutes were conducted by using a Taq DNA polymerase (manufactured by TAKARA EX Taq, Takara Shuzo Co., Ltd.) in a thin wall tube. A part of the obtained PCR reaction mixture was analyzed by agarose gel electrophoresis, DNA fragment having about 1.7 kbp was observed. These can be thought to be protoporphyrinogen oxidase cDNA fragment. Accordingly, these DNA was ligated with the plasmid vector pCR™2.1 having T over hang, using Original TA Cloning KIT-TA CLONING kit (manufactured by Invitrogen Co., Ltd.), according to the attached manual. And, with a ligation mixture, the competent cell of *E. coli*, XL-1 Blue strain (manufactured by Stratagene Co., Ltd.) prepared by the CaCl₂ method, was transformed, and spread on LB agar medium containing IPTG, Xgal and ampicillin, and incubated at 37 °C for overnight. After the incubation, some white colonies with cDNA insert were selected, cultured with shaking in LB liquid medium containing ampicillin for overnight, then plasmid DNA was prepared by the alkaline lysis method. The obtained plasmid DNA was digested with restriction enzyme, and *E. coli* having a plasmid in which each cDNA was inserted at the positive orientation against the promotor was selected. Plasmids in which chlorophyll type protoporphyrinogen oxidase cDNA were inserted were named as pCR-HC or pCR-RC (Fig. 2), respectively. Then ~~Then~~, glycerol was added to the final concentration of 15%, and the *E. coli* was stored at -80 °C.